X-ray Diffraction

914-Pos

Small-Angle X-Ray Scattering and Computational Modeling Reveal the Multi-Domain Assembly States of Hck in Solution

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The Src tyrosine kinases are large multi-domain enzymes [SH3-SH2-catalytic domain] involved in cellular signaling. Their ability to alternate between catalytically active (high-regulated) and inactive (down-regulated) states in response to specific signals provides a central switching mechanism in cellular transduction pathways. The activity of Src kinases is controlled by the assembly of this multi-domain enzyme. We propose an approach combining small-angle X-ray solution scattering (SAXS) with coarse-grained simulations to characterize quantitatively the multi-domain assembly states of Hck in solution. First, a basis set comprising a small number (~10) of assembly state ''classes'' is generated by clustering the configurations obtained from extensive coarse-grained simulations of Hck. Second, the average theoretical SAXS profile for each class of assembly state in the basis set is calculated by using the coarse-grained Fast-SAXS method [Yang et al, Biophys. J. 96:4449 (2009)]. Finally, the relative population of the different classes of assembly states is determined by using a Bayesian-based Monte Carlo procedure seeking to minimize the difference between the theoretical scattering pattern and SAXS data. This novel integrated approach linking experimental SAXS data and simulations is able to resolve the states of assembly of multi-domain Hck in solution under various conditions. The analysis reveals a shift in the equilibrium population of the assembly states upon the binding of various signaling peptides binding to the SH2 or SH3 domains. This integrated approach provides a new way to investigate complex multi-domain assemblies in solution.

915-Pos

X-Ray Structure of an Amyloid-Oligomer-Specific Monoclonal Antibody Fab

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The reactivity of amyloid-oligomer specific antibody, A11, towards soluble oligomer aggregates formed by proteins with varying sequences suggests that it recognizes a generic, sequence independent epitope that is shared among these soluble oligomers. This epitope appears to be associated with soluble amyloid oligomers and not with amyloid fibrils or natively-folded proteins. Currently, the detailed structure of the soluble oligomers of amyloid beta (Aβ) peptides and other amyloid-related proteins and the epitope or structural motif on soluble oligomers that these conformation-specific antibodies recognize are unknown. Co-crystallization of oligomers in complex with the antigen-binding fragments (Fab) of oligomer-specific antibodies may be a promising approach to study the structure of these oligomers due to the binding specificity of antibodies to their antigen and the crystallizability of the antigen-binding fragments (Fab) of antibodies. 48, 55, 204, and 205 (subtypes of A11) are rabbit monoclonal immunoglobulin Gs (IgGs) that show reactivity for generic oligomer epitopes. Here, the x-ray structure of 204Fab at 1.6 Å resolution is reported. The structure of the apo complementarity-determining regions (CDR) of 204Fab may provide insights into how this conformation-specific antibody recognizes Aβ oligomers. This is also the first reported structure of a rabbit Fab, and it reveals a novel interdomain disulfide bond. Crystallization trials of the Fab and ScFV (Single-chain Variable Fragments) of other oligomer-specific monoclonal antibodies and the co-crystals with oligomers are under way. If co-crystallization of the complex made of the amyloid oligomer and oligomer-specific antibodies are successful, the structural information gained from these studies may contribute to the greater understanding of the molecular mechanism of the toxicity associated with the soluble oligomers. Supported by NIH AG00538, the Cure Alzheimer Fund and a grant from the Larry L. Hillblom Foundation.

916-Pos

X-Ray Crystallography Refinement as Ewald Intended: From Drug Design to Ribosome Crystals

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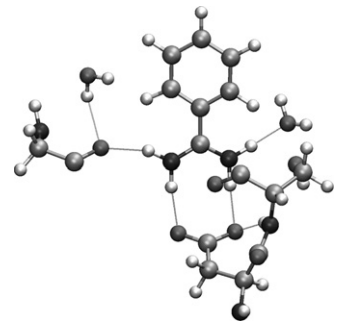
Precise protein-ligand complexes determined via X-ray crystallography experiments are critical for rational drug design. Here we demonstrate both improved statistical precision and greater chemical information content for a range of systems beginning with the trypsin-benzamidine complex shown below and scaling up to ribosome refinements. Keys to our method include use of the AMOEBA polarizable atomic multipole force field combined with a space group particle mesh Ewald (SG-PME) electrostatics algorithm that exploits space group symmetry to increase speed and reduce memory requirements. At high resolution (better than 1.0 Å), we achieve lower R-free statistics by using the AMOEBA electron distribution within the X-ray scattering model. At all resolutions, rigorous treatment of polarizable atomic multipole electrostatics via SG-PME improves molecular conformation and orients the water hydrogen bonding network.



917-Pos

Pair-Distance Distribution Function P(R) of Protein Solution at Crystallographic Resolution

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The pair-distance distribution function *P(r)* is a measure of the frequency of interatomic vector lengths within a protein molecule, and can provide information about the shape and intra-structure of the scattering particle. *P(r)* function has long been recognized as a smooth curve, and the oscillation at low distance is mainly due to unphysical truncation ripples in Fourier transformation.

Recently, we have developed an effective method and succeeded in collecting high-quality SAXS and WAXS of protein solutions, showing negligible radiation damage (1) (2) (3). A high resolution pair-distance distribution function, *P(r)*, of protein molecules has been obtained from the complete solution scattering curve made by combining accurate small-angle and wide-angle X-ray scattering data out to crystallographic resolution (2 Å).

Both indirect and direct Fourier transforms exhibit two distinct peaks at 1.4 Å and 5.1 Å in *P(r)*. X-ray crystallographic data demonstrate that these peaks correspond to two intramolecular distances: the average bond length between C, N and O atoms and the pitch of an α-helix, respectively. Hence some high resolution aspects of the structure and function of a protein can be investigated in solution.

Reference:
1. X. Hong and Q. Hao. 2009. Measurements of accurate x-ray scattering data of protein solutions using small stationary sample cells. Review of Scientific Instruments 80:014303.
2. X. Hong and Q. Hao. 2009. High resolution pair-distance distribution function P(r) of protein solutions. APPLIED PHYSICS LETTERS 94:083903-083903.
3. X. Hong and Q. Hao. 2009. Combining solution wide-angle X-ray scattering and crystallography: determination of molecular envelope and heavy-atom sites. Journal of Applied Crystallography 42:259-264.

Imaging & Optical Microscopy I

918-Pos

Around-the-Objective Total Internal Reflection Fluorescence Microscopy

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Total internal reflection fluorescence (TIRF) microscopy uses the evanescent field on the aqueous side of a glass/aqueous interface to selectively illuminate fluorophores within ~100 nm of the interface. Applications of the method include epi-illumination TIRF where the exciting light is refracted by the microscope objective to impinge on the interface at incidence angles beyond critical angle and prism based TIRF where exciting light propagates to the interface externally to the microscope optics. The former has higher background autofluorescence from the glass elements of the objective where the exciting beam is focused and the latter does not collect near-field emission from the fluorescent sample. Around-the-objective TIRF developed here creates the evanescent field by conditioning the exciting laser beam to propagate through the sub millimeter gap created by the oil immersion high numerical aperture objective and the glass coverslip. The approach eliminates background light due to the admission of the laser excitation to the microscopic optics while collecting near-field emission from the dipoles excited by the evanescent field. The aoTIRF technique was tested with 40 and 100 nanometer diameter fluorescent spheres in water that were diffusing into and out of the detection volume, BFP imaging of single 100 nm fluorescent stationary spheres adsorbed to the glass substrate of the interface, and imaging of cardiac papillary muscle fibers with exchanged GFP tagged myosin ventricular RLC. Results confirm that the evanescent field has penetration depth of ~50 nm, that the background autofluorescence from the